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Negative regulation of P element excision by the somatic product and terminal sequences of P in *Drosophila melanogaster*

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Summary. A transient in vivo P element excision assay was used to test the regulatory properties of putative repressor-encoding plasmids in *Drosophila melanogaster* embryos. The somatic expression of an unmodified transposase transcription unit under the control of a heat shock gene promoter (phsπ) effectively repressed P excision in a dose-dependent manner at very low concentrations relative to somatically active transposase (encoded by the hsπΔ2–3 gene). Maximum repression required transcription of the complete transposase gene. Dose-dependent repression of P excision was also observed in the presence of a vector plasmid (pCarnegie4) having only the terminal sequences, including transposase binding sites, of the P element. However, repression required considerably higher concentrations of pCarnegie 4 than phsπ, and elimination of P excision was not observed.

Key words: P element – Transposable elements – Transposon regulation – *Drosophila melanogaster*

Introduction

The mobilization of transposable elements within the genome of an organism can cause mutations, chromosome rearrangements, altered gene expression, sterility and in some cases death of the organism (see Berg and Howe 1989). Given this potential it is reasonable that the movement of many transposable elements is strictly regulated within the host. In this report we describe the results of experiments designed to elucidate some of the mechanisms responsible for controlling the mobility of the transposable element P in *Drosophila melanogaster*.

P elements, when mobilized, are responsible for the syndrome known as hybrid dysgenesis in *D. melanogaster* (Kidwell et al. 1977; Rubin et al. 1982; Bingham et al. 1982; for a review see Engels 1989). This syndrome includes gonadal dysgenesis, sterility, mutations and chromosome breakage. Two features of the P element transposon system indicate that the regulation of hybrid dysgenesis is complex. First, hybrid dysgenic effects are confined to the germline, and second, hybridization between certain strains, such as P strains which contain P elements and M strains which do not, results in pronounced hybrid dysgenesis only when P strain males are crossed to M strain females. Hybrid dysgenesis is usually not observed following the reciprocal cross (M male × P female) nor between P strains (Kidwell et al. 1977).

The germline specificity of P element mobility results from the cell-type specific removal of the third intron from the primary P element transposase transcript (Laski et al. 1986). Complete or autonomous P elements are 2.9 kb in length, containing four open reading frames essential for autonomous transposition, and three introns (O'Hare and Rubin 1983; Karess and Rubin 1984). In the soma only introns I and II are removed from the primary transposase transcript resulting in the production of a truncated, 66 kD transposase-like protein, while in the germline all introns are spliced leading to the production of functional (87 kD) transposase (Rio et al. 1986). Until recently the function, if any, of the 66 kD protein was unknown.

The nonreciprocal nature of P–M hybrid dysgenesis suggested that the regulation responsible for repression of P mobility in P strains is inherited maternally. This possibility was investigated by Engels (1979) who described a repressive, maternally inherited, cellular state associated with P strains, known as P cytotype, and a permissive cellular state called M cytotype. Although the precise nature of the repressor(s) responsible for P cytotype has not been determined, it is known that the presence of chromosomal P elements is essential for its establishment and maintenance (Sved 1987). Because of the relatively small size of P elements and the presence of only four open reading frames, it was postulated that the repressor might result from the expression of structurally altered P elements producing truncated trans-

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posase-like molecules (O'Hare and Rubin 1983; Karess and Rubin 1984). These proteins would be incapable of catalyzing the movement of P, but they might retain certain functions such as the ability to bind specific DNA sequences, leading to repression of transposition due to competition with functional transposase (Rio et al. 1986). Recently, evidence has been reported that supports this hypothesis (Black et al. 1987; Nitasaka et al. 1987; Nitasaka and Yamazaki 1991), and specifically, that the 66 kD protein is involved in the negative regulation of P mobility (Robertson and Engels 1989; Misra and Rio 1990).

Another type of negative regulation distinct from that proposed for P cytotype was recognized by Simmons and Bucholz (1985). They suggested that the weak P cytotype observed in an M′ *Birmingham* subline might be due to a titration effect caused by the binding of functional transposase to defective P elements. Thus, less transposase would be available to functional P elements, thereby limiting their frequency of mobility.

Results presented here from transient expression P excision assays in embryos have indicated that the somatic expression of the complete transposase transcription unit, as well as the presence of excess numbers of P termini, both result in the repression of P element mobility.

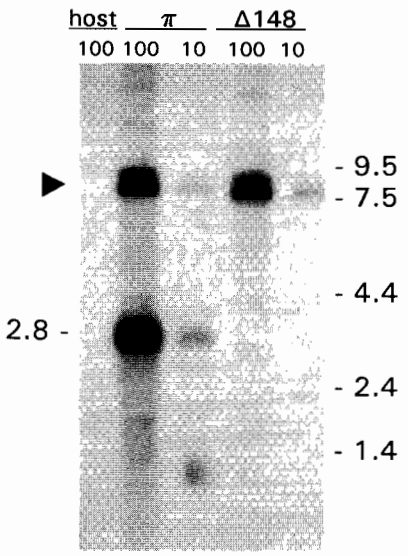

Fig. 1. RNA hybridization blot of P element DNA to total RNA extracted from uninjected *Adh* M strain embryos (host), *Adh* M strain embryos injected with 500 μg/ml phsπ (π), or *Adh* M strain embryos injected with 500 μg/ml phsπΔ148 (Δ148). The indicated plasmid DNA was injected and transiently maintained as described for the excision assay, with RNA extracted at the end of incubation. The number of embryo-equivalents of RNA loaded is listed above each lane. RNA kilobase markers are listed on the sides. The hybridization probe was an antisense RNA sequence complementary to the first 700 bp of the transposase transcript (see Materials and methods for details). The *arrow* indicates a high molecular weight signal due to plasmid DNA recovered in the RNA extraction

Material and methods

Drosophila strains. The genetic symbols used to describe the following strains are explained in Lindsley and Zimm (1992).

Adh^{fn23}*cn*;*ry*^{506}: This M cytotype-containing strain has been used as a host to assess P mobility using a transient in vivo assay (O'Brochta and Handler 1988). It is referred to in this report as the *Adh* M strain.

P[*ry*$^{+}$Δ*2–3*](*99B*): This strain contains two copies of a P element lacking the third intron and inserted at chromosome position 99B (Laski et al. 1986; Robertson et al. 1988; H. Robertson, personal communication). The P element in this strain is stable yet can cause the mobilization of other elements both in the germline and soma at high frequencies (H. Robertson, personal communication).

Plasmids. pISP–2: This P element excision indicator plasmid, constructed by Rio et al. (1986) to assess P transposase activity in cell lines, contains a 600 bp nonautonomous P element originally isolated from the *white* locus of *D. melanogaster* and inserted together with 50 bp of flanking *white* gene sequences into the multicloning site of pUC8. Excision of P from pISP–2, in some cases, restores the LacZα peptide-complementing function encoded by pUC8.

phsπ: This plasmid contains the entire P element transcription unit, including all open reading frames and intervening sequences, under the control of the hsp70 promoter (Steller and Pirrotta 1986). It has been used as a "helper" plasmid in *D. melanogaster* germline transformation experiments to produce functional transposase in the germline (Steller and Pirrotta 1986; D.A. O'Brochta and A.M. Handler, unpublished results) and is transcriptionally active under the transient expression conditions described (Fig. 1).

pUChsπΔ2–3: This plasmid was constructed and kindly provided by F. Laski (University of California, Los Angeles). It was constructed by inserting the 4.6 kb *Bam*HI fragment of pCNΔ2–3 (Rio et al. 1986) into the *Bam*HI site of pUC8. This plasmid contains the P element transcription unit lacking the third intron, under the control of the hsp70 promoter. Its expression in the soma of an M-strain embryo results in the mobilization of P from pISP–2 (O'Brochta and Handler 1988).

phsπΔ148: This plasmid was derived from phsπ by creating a 148 bp deletion in the hsp70 fragment removing 117 bp of the 5′ promoter sequence, including the TATA-like region, and the first 31 bp of the adjacent transcriptional unit. The deletion was created by digesting phsπ with *Nru*I, 31 bp 3′ to the hsp70 transcription initiation site, followed by *Bal*31 digestion and religation. Reaction conditions and sequence analysis were similar to those described by Sambrook et al. (1989). P element transcripts could not be detected in embryos in which phsπΔ148 was transiently expressed (Fig. 1).

pKπAcc: This plasmid was constructed by isolating the 2.4 kb *Acc*I fragment from phsπ, filling-in the resulting terminal 5′ overhang and inserting it into the *Hinc*II site of pK19 (Pridmore 1987). *Acc*I cuts the P element at position 52, within the TATACA sequence approximately 35 bp 5′ to the transcription initiation site (O'Hare and

Rubin 1983; Karess and Rubin 1984), and at position 2411 in the middle of ORF3 (O'Hare and Rubin 1983). The *Acc*I fragment does not contain the P element terminal inverted repeats and lacks most of the putative promoter region.

pCarnegie 4: This P element transformation vector was constructed by Rubin and Spradling (1983) and contains the intact P element termini and flanking *white* gene sequences, however, most of the transcription unit has been eliminated.

P element repressor assay. The in vivo P repressor assay used in this study is a modification of the P excision assay described by O'Brochta and Handler (1988) and is illustrated in Fig. 2. Briefly, the assay involves the transient expression of plasmids in insect embryos, which allows the excision activity of P element sequences to be monitored. The plasmids include a P element excision indicator plasmid (pISP–2) used to report P mobilization, a plasmid encoding somatically active transposase (pUChsπΔ2–3), and a plasmid encoding a putative repressor. All three plasmids were injected into *Adh* M strain embryos, whereas the host strain *P[ry⁺Δ2–3](99B)*, which has a stable chromosomal integration of the somatically active transposase gene, was only injected with pISP–2 and repressor. Plasmids (approximately 10^8 at a DNA concentration of 1 mg/ml) were injected into preblastoderm embryos which were allowed to develop for 18 to 24 h at 24° C, up to the end of embryogenesis, whereupon the embryos were heat shocked at 37° C and plasmid DNA was recovered. P excision from pISP–2 was monitored as a function of restoration of lacZα complementing ability. Ten embryo equivalents of low molecular weight DNA were used to transform 0.2 ml of transformation-competent DH5α *Escherichia coli* (Hanahan 1983). Transformants were plated on LB plates containing ampicillin (75 μg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; 40 μg/ml). One to five thousand ampicillin-resistant transformants per embryo-equivalent of low molecular weight DNA were recovered. Recovery of pISP–2 plasmids from injected embryos, and introduction of these plasmids into DH5α, permitted transformants containing pISP–2 plasmids that lacked the P element as a result of precise or nearly precise excision, to be detected as $LacZ^+$ (blue) colonies on X-gal-containing indicator plates, due to restoration of the lacZα reading frame.

Data and analysis. Excision frequencies were computed by dividing the number of $LacZ^+$ colonies by the total number of pISP–2 plasmids screened (0.9 × ampicillin-resistant $LacZ^+$ and $LacZ^-$ colonies). Final excision frequencies were calculated from the mean ± standard deviation (SD) of weighted frequencies from three to five replicate injection experiments for each plasmid concentration(s) tested. Frequencies were weighted to reflect the differences in the number of plasmids screened in each replicate experiment.

RNA analysis. Total RNA was isolated from uninjected *Adh* M strain embryos or embryos injected with either

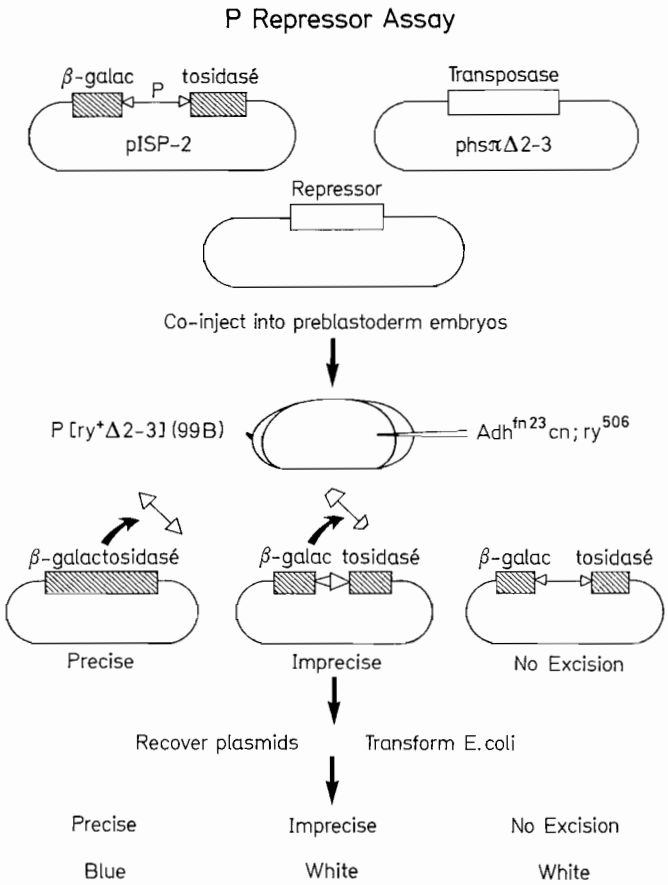

Fig. 2. Diagram of the P element repressor assay. The pUChsπΔ2–3 transposase helper plasmid was only injected into *Adh* M strain embryos. Precise excision refers to those precise or imprecise excision events which result in restoration of the β-galactosidase (lacZα) reading frame. Imprecise excision refers to only imprecise events which do not restore the reading frame. See Materials and methods for details

phsπ or phsπΔ148 plasmid (500 μg/ml), which were heat-shocked prior to isolation. RNA was size-fractionated by agarose gel electrophoresis under denaturing conditions and transferred to nitrocellulose. The filter-bound RNA was hybridized to a uniformly ^{32}P-labelled P-element probe consisting of a 700 nucleotide antisense RNA complementary to the transposase ORF 0 and a portion of ORF 1.

Results

P mobility catalyzed by chromosomal and plasmid-encoded transposase

Robertson et al. (1988) used the hypermutable sn^w allele to demonstrate that flies of the strain *P[ry⁺Δ2–3](99B)* had endogenous levels of functional transposase in the germline comparable to that found in a strong P strain. Furthermore they detected mosaic patches of sn^+ and sn^e bristles in sn^w, ry^{506} males containing *Pry⁺Δ2–3(99B)*, indicating that somatically active transposase was produced. Figure 3A shows that functional transposase

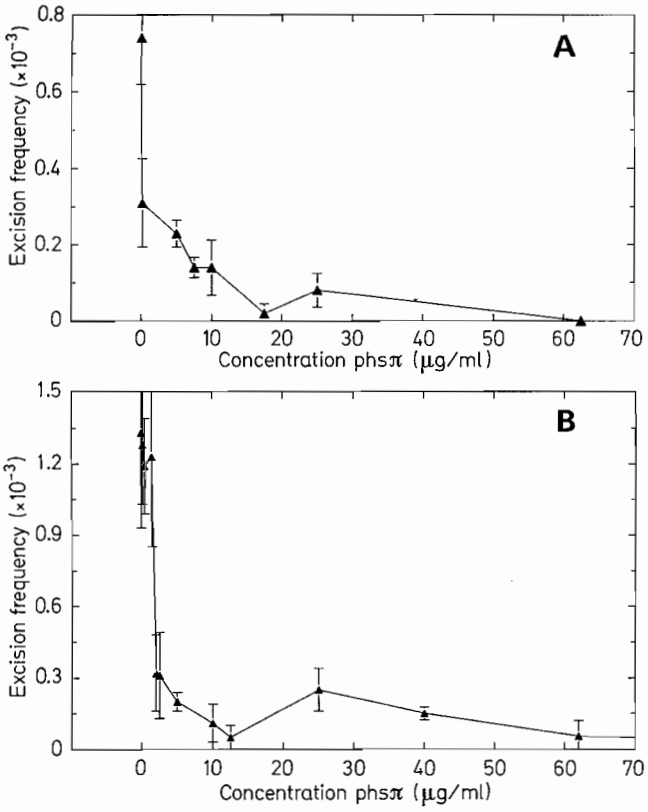

Fig. 3A, B. Excision frequencies (mean ± SD) in **(A)** *P[ry⁺Δ2–3](99B)* embryos injected with 500 μg/ml pISP–2 and indicated concentrations of phsπ, and **(B)** *Adh^fn23 cn; ry^506* M strain embryos injected with 500 μg/ml pUChsπΔ2–3 and pISP–2, and indicated concentrations of phsπ. Higher concentrations of phsπ tested in both strains (up to 500 μg/ml phsπ) yielded no excision events. Excision frequencies represent the accumulated data from 3 to 5 separate injection experiments with $4–10 \times 10^5$ pISP–2 plasmids screened in total for each data point

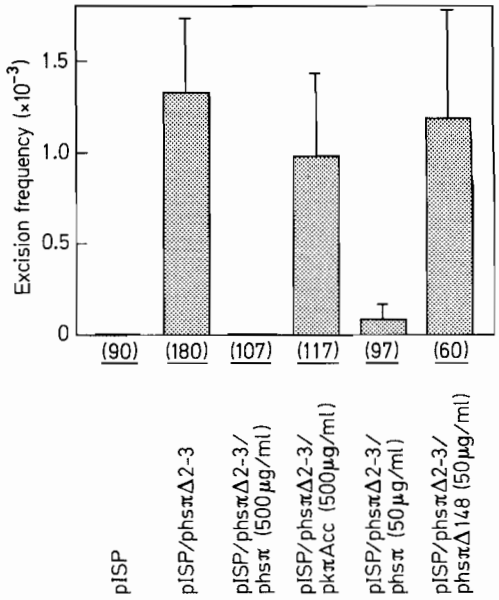

Fig. 4. Excision frequencies (mean ± SD) after injection and transient expression of the indicated plasmids in the M strain *Adh^fn23 cn; ry^506*. The number (in thousands) of pISP–2 plasmids screened from 3 to 5 separate injection experiments are given below the bars. Plasmid concentrations in all experiments for pISP–2 and phsπΔ2–3 was 500 μg/ml. Other plasmids injected are listed with their injected concentration

is also produced in the embryonic soma of *P[ry⁺Δ2–3](99B)*. Injection of the plasmid pISP–2 (at 500 μg/ml; zero concentration phsπ) and subsequent plasmid isolation 24 h later resulted in the recovery of P element excision products at a frequency of 0.74×10^{-3}.

Similar experiments in which M strain embryos (*Adh*) were injected with pISP–2 (500 μg/ml) and the pUChsπΔ2–3 plasmid (500 μg/ml) resulted in a frequency of excision of 1.33×10^{-3} (Figs. 3B and 4), similar to that achieved previously (1.7×10^{-3}; O'Brochta and Handler 1988), but approximately two-fold higher than that observed in the strain *P[ry⁺Δ2–3](99B)*. The differences in excision frequency observed between these two experiments may reflect quantitative differences in transposase transcriptional activity between the plasmid-borne hsπΔ2–3 gene, and the chromosomal copy of πΔ2–3 which is not under hsp70 promoter control, as well as the large number of transposase genes provided by plasmid injection.

Repression by the somatic expression of phsπ

The plasmid phsπ contains the entire P element transcription unit under hsp70 promoter control which resulted in the production and accumulation of 2.8 kb transcripts in the soma (Fig. 1). These incompletely processed transcripts presumably contain the third intron and encode a 66 kD protein resembling P transposase, but which is incapable of mediating the mobilization of P (Laski et al. 1986; Rio et al. 1986; Misra and Rio 1990). Injection of *P[ry⁺Δ2–3](99B)* with the indicator plasmid pISP–2 (500 μg/ml) and the putative repressor-encoding plasmid phsπ at concentrations ranging from 0.2 to 500 μg/ml reduced the frequency of excision in a concentration-dependent manner (Fig. 3A). A significant level of repression (0.31×10^{-3} excision frequency) was observed at the lowest phsπ concentration tested (0.2 μg/ml), with a basal level of repression (greater than a 10-fold decrease in excision) achieved between approximately 10 and 18 μg/ml phsπ. Maximal repression (no excision) was observed at input concentrations of 62 μg/ml phsπ and higher.

The repressor properties of phsπ were also demonstrated following the coinjection of *Adh* M strain embryos with the excision indicator pISP–2, the helper plasmid pUChsπΔ2–3, and the putative repressor phsπ. As with *P[ry⁺Δ2–3](99B)*, repression was concentration dependent with a rapid decrease in excision activity at relatively low levels of phsπ (Fig. 3B). Despite the presumed higher level of transposase due to the heat shock promoter and the large number of genes injected, the effective concentrations of phsπ necessary to reach a basal level of repression, as well as full repression, were similar to those observed in *P[ry⁺Δ2–3](99B)*.

The dependence of repression on phsπ transcription was determined by deletion of the phsπ promoter region. Two plasmids, phsπΔ148 and pKπAcc, were created which had undetectable levels of transcription (Fig. 1;

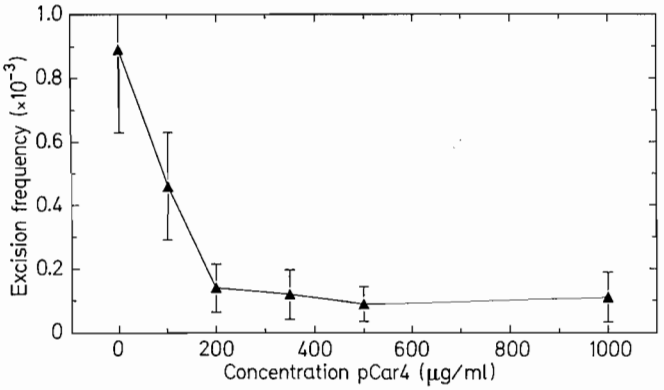

Fig. 5. Excision frequencies (mean ± SD) in *P[ry⁺Δ2–3](99B)* embryos injected with 500 μg/ml pISP–2 and indicated concentrations of pCarnegie4. Excision frequencies represent the accumulated data from at least three separate injection experiments with 5–10 × 10^4 pISP–2 plasmids screened in total for each data point

data not shown for pKπAcc). Testing of these plasmids at two different concentrations revealed no repressor activity compared to the same concentrations of phsπ (Fig. 4) indicating that phsπ transcription was required for maximum repression of P element excision. These experiments also serve as a control for possible inhibition of P excision due to high DNA concentrations injected or non-promoter phsπ sequences.

Repression by P terminal sequences

The reduced levels of P excision observed in certain strains of *D. melanogaster* carrying numerous copies of defective P elements has been suggested to result from the binding and titration of available transposase by nonautonomous P elements (Simmons and Bucholz 1985). To directly test this model P excision was monitored in *P[ry⁺Δ2–3](99B)* embryos following the injection of the pISP–2 indicator plasmid and pCarnegie4 plasmids, which contain only the terminal sequences of P with the presumed transposase binding sites. In this experiment excision frequencies decreased in a concentration-dependent manner, with 50% repression occurring at 100 μg/ml pCarnegie4, and basal levels of excision being reached at approximately 200 μg/ml (Fig. 5). Whereas phsπ completely repressed P excision at concentrations above 62 μg/ml, complete repression was not observed with pCarnegie4 concentrations as high as 1 mg/ml.

Discussion

Transposable elements are dynamic components of genomes whose movement can effect the fitness of an organism and provide a source of variation (Berg and Howe 1989). In addition, they can also be used as vectors to introduce DNA into a genome (Rubin and Spradling 1982). Understanding the mechanisms responsible for regulating transposable element movement is critical to understanding the evolution of genomes that contain transposons, and to the development of transposon-derived insect gene vectors that are phylogenetically unrestricted (O'Brochta and Handler 1988; Handler and O'Brochta 1991). This study presents evidence which supports two models of negative regulation on P mobility – the effects of a truncated somatic product of the transposase gene and transposase terminal sequences remaining in nonfunctional P elements. These studies also reveal an approximate quantitative relationship among the factors involved.

Repression by a truncated translational product of P

Genetic analysis of hybrid dysgenesis in *D. melanogaster* has suggested that P factors encode a negative regulator as well as a positively-acting transposase. This was based on the observation that a P cytotype developed when P-containing chromosomes were introduced into an M strain (Engels 1979; Kidwell 1981) and disappeared when P-containing chromosomes were removed (Sved 1987). Unfortunately the isolation and sequencing of autonomous P elements yielded few clues as to the nature of the P repressor because it appeared that there was only a single transcription unit including four ORFs, all of which are required for the mobility of P (O'Hare and Rubin 1983). Sequence analysis revealed a region of the transposase gene resembling a DNA-binding domain and it was suggested that the 66 kD protein, produced as a result of incomplete processing of the primary transcript, and containing the binding region, might function as a negative regulator (Rio et al. 1986).

Some evidence for the biochemical role of the 66 kD protein and other products of modified P elements in the regulatory process has been reported. Sakoyama et al. (1985) reported the existence of a Q strain (a strain with a repressive cytotype but incapable of inducing hybrid dysgenesis) which appeared to lack complete P elements. However, the structure of the elements responsible for the repressive cytotype in this strain were not identified.

Nitasaka et al. (1987) correlated the existence of a P cytotype with the presence of a defective P element containing a deletion between positions 1991 and 2448 (O'Hare and Rubin 1983), removing a portion of the third intron and half of the fourth open reading frame. Based on the structure of this element, it was predicted that it would result in the production of a protein identical to the transposase-like 66 kD protein produced in the soma plus an additional six amino acids. The authors concluded that the fourth open reading frame (ORF 3) was unnecessary for the suppression of P mobility and that a single copy of the Δ1991–2448 P element could completely repress transposase activity. More recent evidence indicates that the repressor properties of this element were not tested in isolation from other elements, suggesting that other deletion derivatives of P are involved in the determination of P cytotype (Nitasaka and Yamazaki 1991).

Black et al. (1987) reported the identification of another repressor-encoding P element in which nucleotides 808–2560 are deleted. This element (KP), which

produces a 0.8 kb poly(A)$^+$ RNA transcript, was correlated with the repression oberved in certain M′ strains. As with the element described by Nitasaka et al. (1987), the repressor properties of KP have not been tested in isolation from other P elements.

Finally, Robertson and Engels (1989) and Misra and Rio (1990) have identified modified P elements that encode repressors of P mobility. Robertson and Engels (1989) found two elements, the first containing a frameshift mutation at the *SalI* site in the fourth open reading frame and the second containing a mutation in the 3′ splice junction of intron III. When expressed in the soma, both elements were expected to produce the usual 66 kD somatic transposase product. Depending on their genomic location, both elements repressed somatic P mobility (Robertson and Engels 1989). Similarly, Misra and Rio (1990) tested the ability of a P element, containing a deletion which removed half of the third intron and the fourth open reading frame, to repress P mobility in the germline and soma. As observed by Robertson and Engels (1989), this element was expected to produce the 66 kD product. While repression was detected, the level observed appeared to be a function of the genomic location of the repressor.

In this study a transient in vivo P element excision assay involving the coinjection and expression of a P excision indicator plasmid, a plasmid or chromosomal gene encoding somatically active transposase, and a plasmid encoding a putative repressor, was used to test directly the regulatory properties of a variety of P element-derived plasmids. The assay permitted the testing of putative repressor-encoding plasmids in isolation from other elements and independent of their genomic location. The results demonstrated that somatic expression of the transposase gene encoded by phsπ resulted in the repression of P mobility. We found that the level of repression was dependent on the concentration of phsπ initially injected and maximal repression was transcription dependent. The level of injected phsπ that was effective in significantly repressing P mobility (80% repression with 2 μg/ml phsπ) in the M strain was 250-fold lower than the injected concentration of the transposase-encoding helper plasmid pUChsπΔ2–3 (500 μg/ml). If repression is occurring due to the truncated transposase molecule having a P binding domain which competes for binding with the functional molecule, then a higher binding affinity or a non-competitive binding mode may be inferred for the repressor. Experiments designed to measure directly the amounts of repressor and transposase in cells in which P mobility has been repressed are needed to resolve this possibility definitively.

Recent biochemical and genetic evidence indicate that both the complete 87 kDa transposase product (Kaufman and Rio 1991), as well as truncated transposase products (Lemaitre and Coen 1991), have a negative influence on transposase transcription. This is presumably due to the proximity of the transposase and RNA polymerase complex binding sites. In our experiments with *P[ry$^+$Δ2–3](99B)*, which has a normal P promoter, we cannot rule out the possibility that the transposase products inhibited normal transposase transcription. This possibility is less likely in experiments with pUChsπΔ2–3 where regulation by the transposase promoter is superseded by the heat-shock promoter, whose RNA polymerase complex binding site is approximately 250 bp a way from the transposase binding site. Nevertheless, the formal possibility exists that downstream transposase binding influences heat shock driven transcription in this construct.

Repression by titration of transposase by P termini

An alternative model of P regulation has been proposed which does not require a P-encoded regulator. Simmons and Bucholz (1985) suggested that nonautonomous P elements could repress P mobility by binding available transposase, thereby lowering the concentration of transposase available for interaction with functional P elements. Our data supports the notion that P termini can repress P mobility, although repression by pCarnegie4 was considerably less efficient than that associated with the truncated transposase molecule. Fifty per cent repression by pCarnegie4 in the *P[ry$^+$Δ2–3](99B)* strain required an approximately 500-fold higher concentration of plasmid relative to phsπ. While our data does not directly specify mechanisms, apparently the two repressor molecules are acting in different ways.

Regulation of P mobility

The regulation of P mobility is under multiple levels of control, the molecular details of which are beginning to be elucidated. P element derivatives containing either internal deletions or mutations that truncate transposase transcripts or proteins result in the repression of P mobility. In this study incompletely processed transposase transcripts, normally produced in the soma of strains containing complete elements, were also able to repress P mobility. While repressors have been identified, none have been shown to be responsible for all of the characteristics of a P cytotype, indicating that other important aspects of P regulation remain to be elucidated. For example, P cytotype may only arise when certain combinations of repressor-producing elements are present. In several studies, following the introduction of a single autonomous P element into an otherwise P-free M strain, it was found that P cytotype was usually slow to develop and rarely was the switch from M to P cytotype abrupt (Daniels et al. 1987; Kidwell et al. 1988; Preston and Engels 1989). The delay in the development of a P cytotype was expected considering that the regulator has to arise de novo, however, the absence of an abrupt switch in cytotype suggests that multiple elements may be involved. Consistent with these observations, we found repression to be dose-dependent, although the concentration of repressor required to influence P excision negatively was significantly less than the concentration of transposase.

In addition to transcription-dependent repression, a reduction in P excision was also observed in the presence

of P termini suggesting that a titration-like effect as described by Simmons and Bucholz (1985) may be involved. The relatively low rate of repression and inability to repress P excision fully by P termini might indicate that competing sites for transposase binding do not by themselves result in P cytotype.

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References

Berg DE, Howe MM (1989) *Mobile DNA*. American Society for Microbiology, Washington, DC

Bingham PM, Kidwell MG, Rubin GM (1982) The molecular basis of P–M hybrid dysgenesis: The role of the P element, a P strain-specific transposon family. Cell 29:995–1004

Black DM, Jackson MS, Kidwell MG, Dover GA (1987) KP elements repress P-induced hybrid dysgenesis in *D. melanogaster*. EMBO J 6:4125–4135

Daniels SB, Clark SH, Kidwell MG, Chovnick A (1987) Genetic transformation of *Drosophila melanogaster* with an autonomous P element: phenotypic and molecular analyses of long-established transformed lines. Genetics 115:711–723

Engels WR (1979) Hybrid dysgenesis in *Drosophila melanogaster:* Rules of inheritance of female sterility. Genet Res (Camb) 33:219–236

Engels WR (1989) P elements in *Drosophila*. In: Berg DE, Howe MM (eds) *Mobile DNA* American Society for Microbiology, Washington, DC, pp 437–484

Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. J Mol Biol 166:557–580

Handler AM, O'Brochta DA (1991) Prospects for gene transformation in insects. Annu Rev Entomol 36:159–183

Karess RE, Rubin GM (1984) Analysis of P transposable element functions in *Drosophila*. Cell 38:135–146

Kaufman PD, Rio DC (1991) *Drosophila* P-element transposase is a transcriptional repressor in vitro. Proc Natl Acad Sci USA 88:2613–2617

Kidwell MG (1981) Hybrid dysgenesis in *Drosophila melanogaster:* The genetics of cytotype determination in a neutral strain. Genetics 98:275–290

Kidwell MG, Kidwell JF, Sved JF (1977) Hybrid dysgenesis in *Drosophila melanogaster:* A syndrome of aberrant traits including mutation, sterility, and male recombination. Genetics 86:813–833

Kidwell MG, Kimura K, Black DM (1988) Evolution of hybrid dysgenesis potential following P element contamination in *Drosophila melanogaster*. Genetics 119:815–828

Laski FA, Rio DC, Rubin GM (1986) Tissue specificity of *Drosophila* P element transposition is regulated at the level of mRNA splicing. Cell 44:7–19

Lemaitre B, Coen D (1991) *P* regulatory products repress in vivo the *P* promoter activity in *P-lacZ* fusion genes. Proc Natl Acad Sci USA 88:4419–4423

Lindsley DL, Zimm GG (1992) *The Genome of Drosophila melanogaster*. Academic Press, San Diego

Misra S, Rio DC (1990) Cytotype control of *Drosophila* P element transposition: The 66 kd protein is a repressor of transposase activity. Cell 62:269–284

Nitasaka E, Mukai T, Yamazaki T (1987) Repressor of P elements in *Drosophila melanogaster:* Cytotype determination by a defective P element with only open reading frames 0 through 2. Proc Natl Acad Sci USA 84:7605–7608

Nitasaka E, Yamazaki T (1991) Genetic variations and regulation of P elements in *Drosophila melanogaster*. In: Kimura M, Takahata T (eds) New aspects of the Genetics of Molecular Evolution. Japan Sci Soc Press, Tokyo, pp 263–276

O'Brochta DA, Handler AM (1988) Mobility of P elements in drosophilids and nondrosophilids. Proc Natl Acad Sci USA 85:6052–6056

O'Hare K, Rubin GM (1983) Structure of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell 34:25–35

Preston CR, Engels WR (1989) Spread of P transposable elements in inbred lines of *Drosophila melanogaster*. Prog Nucleic Acids Res Mol Biol 36:71–85

Pridmore RD (1987) New and versatile cloning vectors with kanamycin-resistance markers. Gene 56:309–312

Rio DC, Laski FA, Rubin GM (1986) Identification and immunochemical analysis of biologically active *Drosophila* P element transposase. Cell 44:21–32

Robertson HM, Engels WR (1989) Modified P elements that mimic P cytotype in *Drosophila melanogaster*. Genetics 123:815–824

Robertson HM, Preston CR, Phillis RW, Johnson-Schlitz D, Benz WK, Engels WR (1988) A stable genomic source of P element transposase in *Drosophila melanogaster*. Genetics 118:461–470

Rubin GM, Kidwell MG, Bingham PM (1982) The molecular basis of P–M hybrid dysgenesis: The nature of induced mutations. Cell 29:987–994

Rubin GM, Spradling AC (1982) Genetic transformation of *Drosophila* with transposable element vectors. Science 218:348–353

Rubin GM, Spradling AC (1983) Vectors of P element mediated gene transfer in *Drosophila*. Nucleic Acids Res 11:6341–6351

Sakoyama Y, Todo T, Ishiwa-Chigusa S, Honjo T, Kondo S (1985) Structures of defective P transposable elements prevalent in natural Q and Q-derived M strains of *Drosophila melanogaster*. Proc Natl Acad Sci USA 82:6236–6239

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. A laboratory manual. (2nd edn) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Simmons MJ, Bucholz LM (1985) Transposase titration in *Drosophila melanogaster:* A model of cytotype in the P–M system of hybrid dysgenesis. Proc Natl Acad Sci USA 82:8119:8123

Steller H, Pirrotta V (1986) P transposons controlled by the heat-shock promoter. Molec Cell Biol 6:1640–1649

Sved JA (1987) Hybrid dysgenesis in *Drosophila melanogaster:* evidence from sterility and Southern hybridization that P cytotype is not maintained in the absence of chromosomal P factors. Genetics 115:121–127

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